NF-κB/Jurkat/GFP™ Transcriptional Reporter Cell Line

Cat. # TR850A-1

User Manual

Store vial in vapor phase of liquid nitrogen on receipt

(ver. 1-071212)

Contents

I. Introduction and Background

A. Overview

The NF-κB/Jurkat/GFP™ cell line was specifically designed for monitoring the NF-κB signal transduction pathway *in vitro* in a physiologically relevant cell line. The NF-κB/Jurkat/GFP™ cells were derived from the human T lymphocyte-based Jurkat cell line. Jurkat cells were transduced with HIV-based pseudoviral particles packaged with an expression vector encoding the GFP reporter gene driven by the minimal cytomegalovirus (mCMV) promoter in conjunction with four copies of the NF-κB consensus transcriptional response element upstream of mCMV (Figure 1). Positively transduced cells were cloned by Fluorescent Activated Cell Sorting (FACS), and clonal populations that stably retained the proviral expression construct in Jurkat cell genomic DNA over many passages were selected. Clones that demonstrated a robust (≥ 30-fold) increase in GFP expression upon stimulation with TNF-α at 10 ng/ml for 24 hours were chosen for further development of the cell line (Figure 2). The resulting NF-κB/Jurkat/GFP™ cell line is the clonal population that exhibited the lowest background and the strongest response to TNF-α stimulation (Figure 3, page 3).

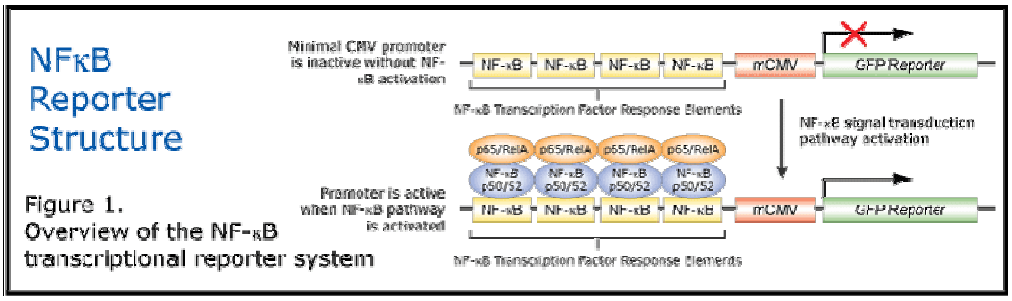

Figure 1. Overview of the NF-κB transcriptional reporter system

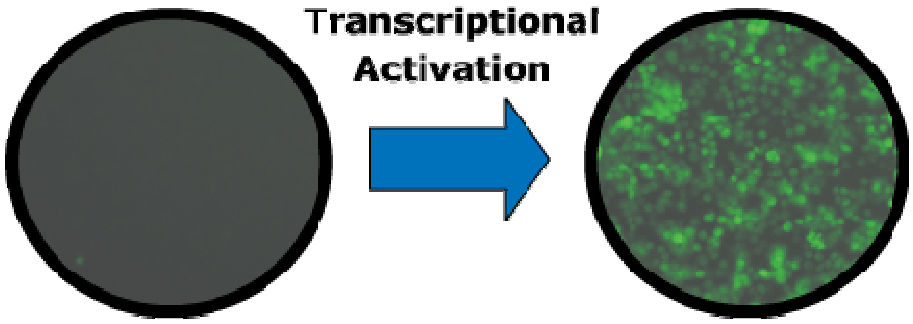

Figure 2. Fluorescent microscopy visualization of acitvated NF-κB/Jurkat/GFP™ cell line

Utilization of the GFP reporter gene allows the researcher to detect NF-κB activation by fluorescence microscopy, and offers the advantage of allowing for GFP-positive or negative cells to be sorted by FACS. As a result, the NF-κB/Jurkat/GFP™ cell line is completely compatible with SBI's HIV- and FIV-based genome-wide siRNA libraries and individual siRNA lentiviral vectors for RNAi knockdown studies, as well as cDNA vectors to identify genes involved in the stimulation or inhibition of the NF-κB pathway. NF-κB/Jurkat/GFP™ cells are transducible human cells which serve as useful *in vitro* cell models for a variety of research applications, including screening of small molecule inhibitors or activators of the NF-κB pathway, and the identification of genes involved in the inhibition or activation of the pathway by use of genome-wide siRNA libraries or specific siRNAs, available from System Biosciences. The data below illustrates activation of the NF-κB/Jurkat/GFP™ cell line with varying concentrations of TNF-α (Figure 3). The GFP signal was also detected using a BioTek Synergy 2 Microplate Reader on live cells (Figure 3 A,B), demonstrating the potential for easy signal measurement in high throughput screens.

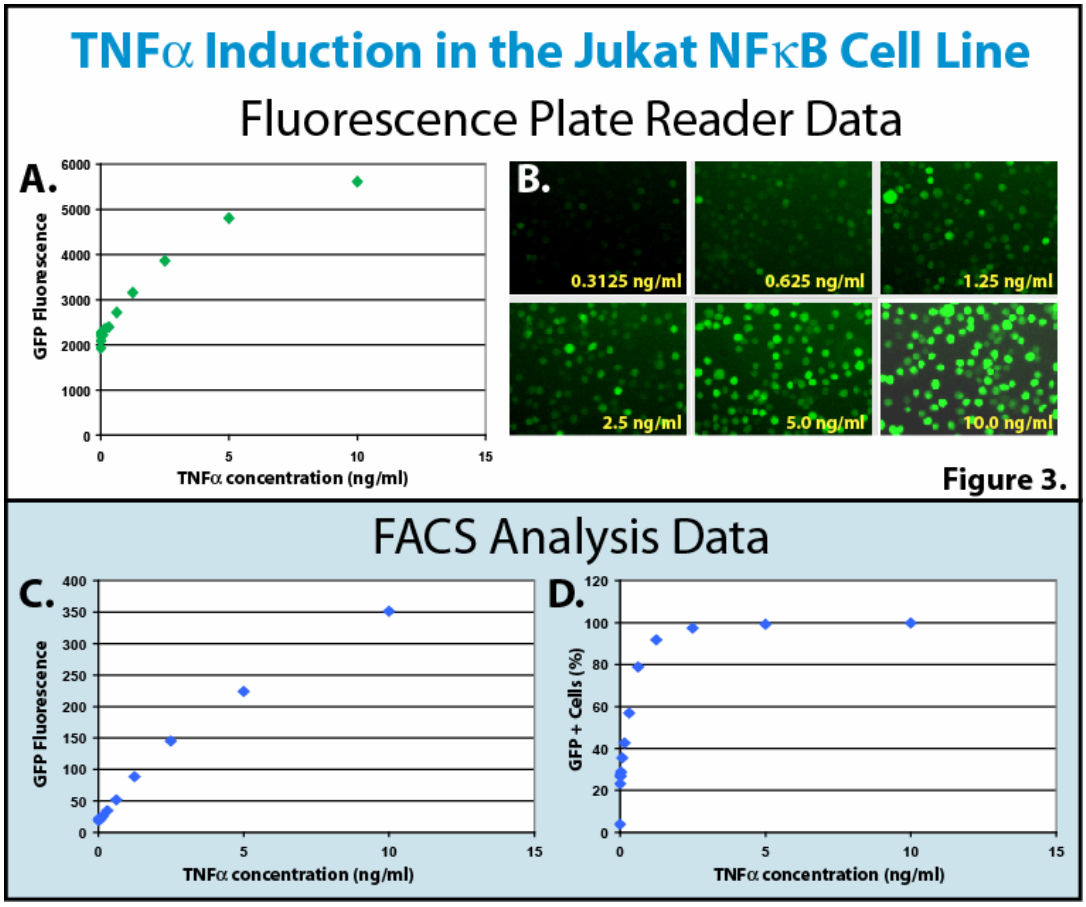

Figure 3. Activation of NF-κB/Jurkat/GFP™ cells with increasing amounts of TNF-a. NF-κB/Jurkat/GFP™ Reporter cells (5x10^5 cells) were plated at a concentration of 1 million cells/ml into each well of a 24-well plate. TNF-α was added in the amount indicated in the Figure. After 24 hours, 100 µl of the cells were transferred to a well of a Costar® UV plate (96 well, No lid, w/ UV Transparent Flat Bottom, Corning, NY, Cat# 3635) and the intensity of GFP fluorescence was measured (Excitation 485+/-20, Emission 528+/-20) in a Synergy™ HT Multi-Detection Microplate Reader (BioTech, Winooski, Vermont). The intensities of GFP measured were plotted against the amount of TNF-α **(A).** In the meantime, the fluorescent cells in the original 24-well plate were photographed on a ZEISS inverted microscope **(B)**. Alternatively, 200 µl of the cells were fixed with 200 µl of 4% formaldehyde and GFP reporter induction analyzed by flow cytometry, and either the GFP intensities **(C)** or the percentage of GFP positive cells **(D)** were plotted against the amount of TNF-α.

B. NF-κB Signal Transduction Pathway

NF-κB, a member of the rel family of transcription factors, regulates several important physiological processes, including immune responses, inflammation, cell growth, apoptosis, tumorigenesis, and the expression of certain virus genes (HIV and CMV). As a result, the NF-κB signaling pathway has been a target for pharmacological intervention, especially in models of inflammation or cancer, where the pathway is often constitutively active (1). Over 750 inhibitors of the NF-κB pathway have been identified, including both natural and synthetic molecules (1). Conversely, many different stimuli have been identified which activate the NF-κB pathway, including cytokines such as TNF-α, and interleukin-1β, pathogenic bacteria and viruses, bacterial lipopolysaccharide and peptidoglycan, and oxidative stress (Figure 4.). The NF-κB/Jurkat/GFP™ cell line allows the researcher to study both potential inhibitors, activators, and antagonists of the NF-κB pathway, and as such, is an extremely valuable tool for a wide variety of research applications.

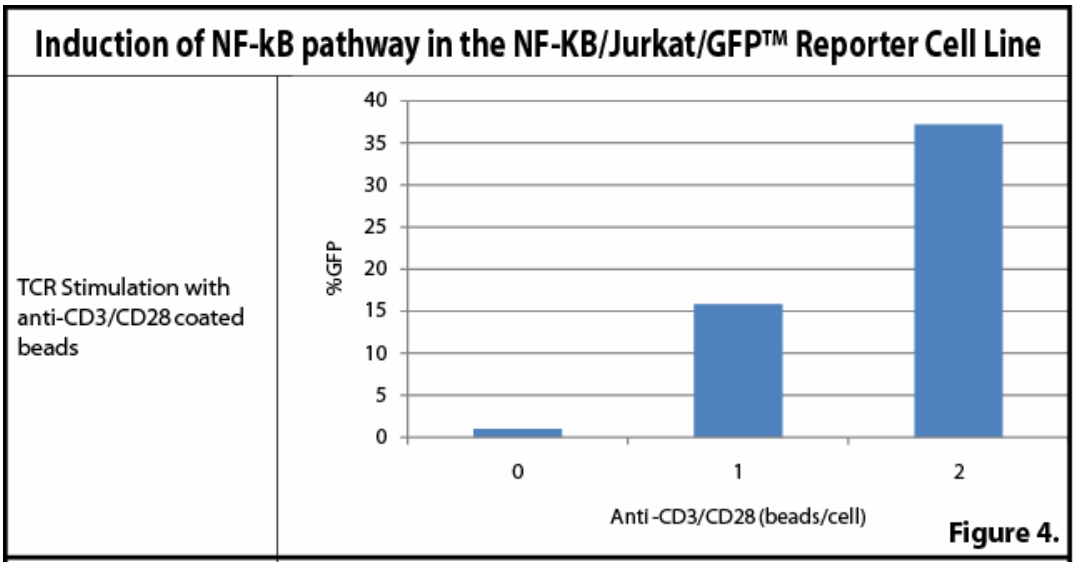

Figure 4.

NF-κB/Jurkat/GFP™ Reporter cells ($5x10^5$ cells) were plated at a concentration of 1 million cells/ml into each well of a 24-well plate. Either Dynabeads® anti-CD3/CD28 TCell Expander (Invitrogen, CA. Cat# 111.31D) or Phytohemagglutinin (PHA, M Form, Invitrogen, Cat# 10576) were added in the amount indicated in the figure. After 24 hours, the cells were fixed in 2% formaldehyde and GFP reporter induction analyzed by flow cytometry.

C. List of Components

NF-κB/Jurkat/GFP™ cells (catalog # TR850A-1) are supplied in one vial containing approximately 2×10^6 cells in 1 ml of freezing medium (complete growth medium w/ 10% Dimethyl sulfoxide, DMSO).

- **1 ml NF-κB/Jurkat/GFP™ cells (~2×10^6 cells)**
- **User Manual**

D. Shipping and Storage

The NF-κB/Jurkat/GFP™ cell lines are shipped frozen on dry ice. It is strongly recommended that the NF-κB/Jurkat/GFP™ cells be thawed and propagated as soon as possible following receipt (see "Thawing NF-κB/Jurkat/GFP™ cells" protocol below). If long-term storage of the frozen cells is required, place vial in the vapor phase of liquid nitrogen. Storage of the cells directly in liquid nitrogen requires use of protective tubing, such as Nunc™ Cryoflex™ Tubing (Mfr. No. 373958). Storage of the cells at -80ºC is suitable only for short periods of time (3-4 days), and may result in loss of viability and is not recommended.

E. Product Qualification

Each lot of NF-κB/Jurkat/GFP™ cells is tested for growth and viability following recovery from cryopreservation. In addition, each lot is tested for expression of Green Fluorescent Protein (GFP) reporter gene following stimulation with 10 ng/ml TNF-α. Only those cells exhibiting ≅ 30-fold average GFP expression compared to unstimulated Jurkat-NF-κB cells pass quality assurance (Figure 5).

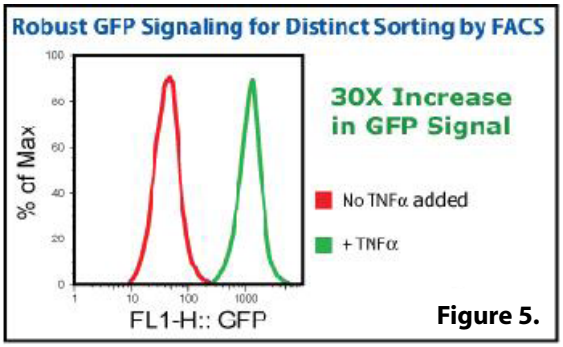

Figure 5.

F. Safety Guidelines

The NF-κB/Jurkat/GFP™ cell line falls within NIH Biosafety Level 2 criteria and should be handled as potentially biohazardous material. This product contains Dimethyl sulfoxide (DMSO), a hazardous material. For a description of laboratory biosafety level criteria, consult the Centers for Disease Control Office of Health and Safety Web site at http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4s3.htm. It is also important to check with the health and safety guidelines at your institution regarding the use of potentially biohazardous cell lines, and always follow standard tissue culture practices, which include:

- Wearing gloves, safety glasses, and a lab coat at all times when conducting the procedure
- Always working with cells in a Class II laminar flow hood
- Carefully performing all procedures to minimize the creation of aerosols or splashes
- Decontamination of all work surfaces at least once a day and immediately after working with cells
- Decontamination of all cultures, viral stocks, and other regulated wastes before disposal by an approved method such as autoclaving

Materials to be decontaminated outside of the immediate laboratory area are to be placed in a durable, leakproof, properly marked (“Biohazard”, “infectious waste”) container and sealed for transportation from the laboratory.

II. Protocols

A. Required Media for NF-κB/Jurkat/GFP™ cells

The table below shows the recommended complete medium and freezing medium for maintenance of NF-κB/Jurkat/GFP™ cells.

Complete Growth Medium	Freezing Medium
RPMI	90% Complete Growth Medium
10% fetal bovine serum (FBS)*	10% DMSO
2 mM L-glutamine	
1% Penicillin/Streptomycin (10,000 I.U. Penicillin; 10,000 μg/ml Streptomycin)	

*FBS does not require heat inactivation to be used with the NF-κB/Jurkat/GFP™ cell line

B. Thawing Cells

Use the following protocol to thaw NF-κB/Jurkat/GFP™ cells to initiate the culture. The initial propagation of cells should be used to generate stocks to be frozen and stored for future use.

1. Remove the frozen vial of cells from liquid nitrogen and quickly thaw them by swirling in a 37ºC water bath. Try to keep the O-ring and cap of the vial out of the water, to prevent possible contamination. Wear eye protection.
2. Before cells are completely thawed, remove from 37ºC water bath and decontaminate outside of the vial with 70% ethanol.
3. Using sterile technique, transfer contents of vial to a 15ml conical centrifuge tube containing 10ml of complete media. Centrifuge 10 minutes at 2,000 rpm. Discard supernatant.
4. Resuspend the cells in 20 ml of complete medium at room temperature and transfer to a T-75 cm^2 tissue culture flask.
5. Incubate the cells at 37ºC, 5% CO_2 and check daily. Split cells as needed to maintain a density of 0.5-1 x 10^6.
6. For the initial culture, it is recommended to archive several frozen stocks and continue to propagate remainder of cells for use in experiments.

Note: Vials inappropriately stored directly in liquid nitrogen without protective tubing, such as Nunc™ Cryoflex™ Tubing, may contain liquid nitrogen. Upon thawing, the liquid nitrogen will quickly convert to the gas phase and may cause the vial of cells to explode. This is a very hazardous situation, and should only be performed using protective gloves and clothing, and a full-face mask. To avoid this situation, store vials only in the vapor phase of liquid nitrogen or use the protective tubing described above if the vial must be stored directly in the liquid phase of liquid nitrogen.

C. Subculturing Cells

When NF-κB/Jurkat/GFP™ cells reach ~1 x 10^6 cells / ml, they are ready to be split and transferred to a new tissue culture flask. This is typically every 2-3 days. Determine viable and total cell counts by use of a hemocytometer chamber or a Coulter Counter. Split cells as needed to maintain a density of 0.5-1 x 10^6 cells / ml.

D. Freezing Cells

Preparation of frozen cell stocks

Before beginning the freezing protocol below, label all cryovials and prepare freezing medium (complete growth medium with 10% DMSO). Keep freezing medium at 4ºC or on ice until ready for use.

1. Culture NF-κB/Jurkat/GFP™ cells to between 0.5-1 x 10^6 cells / ml. Cells must be actively dividing for best results.
2. Centrifuge desired number of cells at 2,000 rpm for 10 minutes.
3. Resuspend cell pellet in complete growth media + 10% DMSO (freezing medium) at 2 x 10^6 cells / ml. Dispense 1 ml aliquots of the cells into cryovials following manufacturer's recommendations.
4. Freeze cells using either a controlled-rate freezing apparatus or manually using a freezing container. The apparatus should provide a controlled freezing rate of 1ºC/minute. Cells should be frozen to -70ºC to -80ºC overnight.
5. Transfer frozen cell stocks to liquid nitrogen storage the following day.

III. References

1. **Egan, L. J. and Toruner, M.** NF-κB Signaling: Pros and Cons of Altering NF-κB as a Therapeutic Approach. 2006; Ann. N.Y. Acad. Sci. 1072: 114–122.

IV. Appendix

A. Related Products

- **GeneNet™ Lentiviral siRNA Libraries**
  (Cat. #s SI202B-1, SI206B-1, SI606B-1, SI222B-1, SI622B-1)
  GeneNet™ siRNA Libraries enable high-throughput gene knockdown studies on a genome-wide basis. You can simultaneously identify multiple genes that alter a specific cellular phenotype—in a single experiment. By merging effector expression libraries with siRNA technology and efficient lentiviral transduction, GeneNet™ siRNA Libraries enable the introduction of many thousands of siRNA molecules into target cells at once. After selection of transduced cells that express a desired phenotype, one can identify gene(s) that control the response. Libraries are available in packaged or plasmid form. For a list of currently available siRNA Libraries, please visit our website at www.systembio.com.

- **Lentivector Packaging Kits**
  For FIV-based Vectors: pPACKF1™ (Cat. # LV100A-1)
  For HIV-based Vectors: pPACKH1™ (Cat. # LV500A-1)
  Unique plasmid mixes that produce all the necessary viral proteins and the VSV-G envelope glycoprotein from vesicular stomatitis virus required to make active pseudoviral particles. Producer Cell Line 293TN (SBI Cat. # LV900A-1) transiently transfected with the packaging plasmids and an HIV-based lentiviral construct produce packaged viral particles containing the lentiviral construct of interest.

- **293TN Human Kidney Producer Cell Line** (SBI, Cat. # LV900A-1)
  For packaging of plasmid lentivector constructs.

- **Packaged Positive Transduction Controls**
  FIV-based: pSIF1-H1-siLuc-copGFP (Cat. # LV201B-1)
  HIV-based: pSIH1-copGFP (Cat. # LV600A-1)
  Packaged Positive control lentivectors allow you to measure transduction efficiency in target cells based on percent of GFP-positive cells. The H1-siLuc lentivector expresses an siRNA targeting Luciferase.

- **shRNA Cloning and Expression Lentivectors** (many)
  These FIV and HIV-based single-promoter shRNA cloning vectors allow you to clone and express shRNA constructs. For a list of currently available vectors, please visit our website at www.systembio.com.

- **cDNA Cloning and Expression Lentivectors** (many)
  These FIV and HIV-based cDNA cloning vectors allow strong and ubiquitous expression of your gene of interest. Choose from copGFP or puromycin selection markers. For a list of currently available vectors, please visit our website at www.systembio.com.

- **PathNet™ Transcriptional Reporter Lentivectors** (many)
  Detect the activation of transcriptional factors (TFs) in a natural chromosomal environment based on a lentivector reporter construct and create stable cell lines. Available in plasmid form or pre-packaged in pseudoviral particles. Choose from copGFP, Luciferase, or β-Gal reporters. For a list of currently available vectors, please visit our website at www.systembio.com.

B. Technical Support

For more information about SBI products and to download manuals in PDF format, please visit our web site:

http://www.systembio.com

For additional information or technical assistance, please call or email us at:

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1616 North Shoreline Blvd.
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Technical Support: tech@systembio.com
Ordering Information: orders@systembio.com

V. Licensing and Warranty Statement

Limited Use License

Use of NF-κB/Jurkat/GFP™ cell line (*i.e.*, the "Product") is subject to the following terms and conditions. If the terms and conditions are not acceptable, return all components of the Product to System Biosciences (SBI) within 7 calendar days. Purchase and use of any part of the Product constitutes acceptance of the above terms.

The purchaser of the Product is granted a limited license to use the Product under the following terms and conditions:

The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use.

The Product may not be resold, modified for resale, or used to manufacture commercial products without prior written consent of SBI.

This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research.

SBI has pending patent applications related to the Product. For information concerning licenses for commercial use, contact SBI.

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Limited Warranty

SBI warrants that the Product meets the specifications described in this manual. If it is proven to the satisfaction of SBI that the Product fails to meet these specifications, SBI will replace the Product or provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to SBI within 30 days of receipt of the Product.

SBI's liability is expressly limited to replacement of Product or a refund limited to the actual purchase price. SBI's liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. SBI does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose.
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